

Coding of Neural Information by Neuroblastoma Cells

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Numerous neuroblastoma clones originating from mouse tumor C1300 were characterized with respect to neuronal properties such as neurotransmitter metabolism, cell receptors and response to transmitters, and action potential reactions. Three types of clones were found with respect to enzymes for transmitter synthesis: clones with choline acetyltransferase activity, which synthesize acetylcholine; clones with tyrosine hydroxylase activity, which synthesize catechols; and clones without these enzyme activities. Cells from each type of clone were recloned approximately 200 cell generations after the initial clones were isolated, and enzyme activities of the sublines were determined. Most subclones resembled the parental type with respect to transmitter synthesis. No stem cells or conversion of cholinergic to adrenergic cells were detected. These results show that genes determining neurotransmitter species can be expressed in dividing cells, that the parental programs of gene expression are inherited, and that dividing cells can be programmed with respect to their ability to communicate with other cells. Characterization of receptors and cell responses to putative transmitters also revealed different phenotypes which were inherited in a clonal fashion.

Two types of action potentials are elicited by electrical stimulation: Na^+ spikes that are inhibited by tetrodotoxin, and Ca^{++} spikes that are insensitive to this toxin. Three types of action potential "ionophores" can be distinguished: "ionophores" for Na^+ , Ca^{++} , or K^+ . Defective cell lines were obtained which lack one or more action potential ionophores.

Many neural properties are expressed conditionally. Populations of cells can be shifted from a poorly differentiated, neuroblast-like state to a well-differentiated, neuron-like state by selecting for nondividing cells, decreasing the rate of cell division, or increasing intracellular levels of cyclic AMP. The specific activities of tyrosine hydroxylase, choline acetyltransferase, and acetylcholinesterase can be increased 100-, 15-, and 50-fold, respectively. Populations of electrically passive cells also can be shifted in synchrony to the electrically excitable state.

Additional cell lines with new neural phenotypes were generated, and

questions of dominance of gene expression and complementation were explored by fusing cells and obtaining hybrid cell lines. The expression of genes for neural properties was found to be dominant with most matings. Approximately 25% of neuroblastoma × L cell hybrid clones expressed the neural phenotype of the neuroblastoma parent 25 to 50 cell generations after fusion. Most of the remaining hybrid clones had specific neural defects. Cell lines with defects in transmitter synthesis, storage, and catabolism; response to neurotransmitters; action potential reactions; and so forth, were generated in abundance.

With other crosses new neural properties were acquired by hybrid progeny. For example, fusion of mouse neuroblastoma cells with rat glioma cells, both lacking choline acetyltransferase, yielded hybrid cell lines with high choline acetyltransferase activity. Some but not all of the cholinergic hybrid progeny also store acetylcholine and have clear vesicles identical in appearance to those found at synaptic junctions. Fusion of mouse neuroblastoma cells which do not synthesize catecholamines with normal sympathetic ganglion cells from mouse embryos yielded a hybrid cell line that synthesizes dopamine and has muscarinic excitatory acetylcholine receptors and both small and large dense-core vesicles. These and other results show that fusion of neuroblastoma cells with cells from the normal nervous system generates hybrid cells with new neural properties not detected with neuroblastoma cells. The new neural phenotypes are inherited and thus are perpetuated in a fairly stable fashion for more than 100 cell generations.

A selection procedure was devised for neurons and related cells that depends on the ability of the cells to synthesize certain species of transmitters. The rationale for selection derives from the fact that tyrosine has long been known to be an essential amino acid for mammals, and that three mammalian enzymes are known to catalyze *in vitro* the synthesis of tyrosine from phenylalanine, i.e., phenylalanine hydroxylase, tyrosine hydroxylase, and tryptophan hydroxylase. The two latter enzymes are found predominantly in neurons in related cells that synthesize either dopamine, norepinephrine, epinephrine, serotonin, or melatonin. Only one of 70,000 uncloned mouse neuroblastoma cells grew well in the absence of tyrosine. No colonies were found with cell lines which lack tyrosine hydroxylase, such as cholinergic neuroblastoma cells, glioma cells, or L-cells. Approximately 50% of the neuroblastoma cell lines that grew well in the absence of tyrosine had tyrosine hydroxylase activity. Some adrenergic cell lines obtained by selection also possessed storage mechanisms for catecholamines. This method of selection coupled with somatic cell hybridization affords a simple, versatile means of generating and selecting cells of neural origin on the basis of cell specificity in synthesizing neurotransmitters.

In collaboration with Dr. Werner Klee, neuroblastoma and various hybrid cell lines were assayed for morphine receptors. One cell line, a neuroblastoma × glioma hybrid, was found which synthesizes stereo-

specific, high-affinity morphine receptors. The average cell has approximately 3×10^5 narcotic receptors. In contrast, the neuroblastoma parent cell line has few morphine receptors, and the receptors were not detected with the glioma parent. Additional studies in collaboration with Drs. Shail Sharma and W. Klee show that morphine inhibits adenylate cyclase activity of cells with morphine receptors but has no effect on adenylate cyclase of cells without these receptors. Thus two forms of adenylate cyclase can be distinguished; one form is sensitive and the other is insensitive to narcotics. Questions pertaining to the mechanism of narcotic addiction and dependence are currently being explored with this system.